Cognitive endophenotypes in impulsivity:

5-HTTLPR influences inhibitory control in healthy subjects

Martin Aker og Anne Marie Hoel

Hovedoppgave ved Psykologisk Institutt

UNIVERSITETET I OSLO

Høst 2009

ABSTRACT

Authors: Martin Aker and Anne Marie Hoel

Title: Cognitive endophenotypes in impulsivity: 5-HTTLPR influences inhibitory control in healthy subjects.

Advisor: Professor Nils Inge Landrø

Background: The serotonin transporter polymorphism (5-HTTLPR), related to serotonin transmission in the brain, has been associated with impulsive behavior. Impulsive behavior and lack of inhibitory control are components in various psychiatric disorders. Although several studies have associated 5-HTTLPR and impulsivity, the literature is ambiguous. By measuring impulsivity with objective behavioural measures, and applying the triallelic classification of genotypes, this study aimed to explore the association between impulsivity and 5-HTTLPR. A subordinate aim was to investigate a possible sex by 5-HTTLPR genotype interaction on impulsivity.

Methods: The data were collected as part of the project "Cognitive control, mood, brain function and genetics in major depressive disorder and healthy people". The authors participated in collection of the data. The principal investigator of this project is Nils Inge Landrø. Participants in this study were 87 healthy adults from 19 to 61 years. Blood samples were drawn from all participants for 5-HTTLPR genotyping. Inhibition and response style were assessed with the stop signal task and the continuous performance test.

Results: Results showed a significant effect of genotype on stop signal reaction time. An unexpected age difference between groups moderated this effect. Assuming dominance of the low expressive alleles, SS and SL groups were collapsed. Reanalysis showed significant effects of genotype and age on stop signal reaction time. No significant effect of genotype was found on response style. No significant interaction between genotype and sex was found. The effect of the short allele on response style showed an opposite pattern in men and women, but this effect was not significant.

Conclusion: The present study links the serotonin transporter polymorphism to inhibitory control. Inhibition is a central component of executive functions and impairment in these functions are associated with various psychological disorders. Through its effect on inhibition, the short allele may constitute a genetic vulnerability for impulse control disorders and depression.

ACKNOWLEDGEMENTS

We would like to thank our advisor, Professor Nils Inge Landrø for support and professional advice during the work on this thesis. We would also like to thank cand.psychol. Ph.D. student Pia Lyche and cand.psychol. Rune Jonassen for their contributions to the study, and for encouragement and advice in the process.

This study would not have been possible without the help from Runa and Kari Bente at the Clinical Chemical Department at Oslo University Hospital, Ullevål, who performed genotyping, and the employees at the Psychopharmacological Department at Diakonhjemmet Hospital who handled blood sampling.

For help with statistical analysis we thank associate professor Pål Ulleberg. We also want to thank Bjørn Hoel for helpful comments, and our fellow students for support and encouragement.

TABLE OF CONTENT

INTRODUCTION

The concept of impulsivity plays an important role in describing human behavior and behavioral tendencies in lay language as well as in academic psychology and psychiatry. The tendency to be spontaneous and impulsive is often regarded positively in everyday situations. This positive view of impulsivity is reflected in Norman's taxonomy (1967) of personality descriptors, where impulsivity is pooled with positive adjectives such as "carefree, playful and zany" (as cited in Goldberg, 1990, p. 1218). Others consider impulsivity more negatively as part of neuroticism (McCrae & Costa, 1997). An analogue to these positive as opposed to negative concepts of impulsivity can be found in Dickman's (1990) work, which distinguishes a functional from a dysfunctional type. According to Dickman, functional "impulsives" are lively, adventurous, and willing to take risks, and appear to have been rewarded for their style. The errors they inevitably sometimes commit as result of acting without forethought seem to be compensated by their productivity. Dysfunctional impulsivity, on the other hand, is the tendency to act with little forethought despite the fact that this frequently leads the individual into difficulties. According to Dickman, these two kinds of impulsivity are weakly related, he therefore describes them as separate and largely unrelated dimensions, rather than closely related traits or poles of one dimension.

Barratt (1985) described impulsiveness with the three dimensions, motor, non-planning, and cognitive impulsiveness. A more recent analysis suggested that cognitive processes concern impulsiveness in general and underlie all dimensions of impulsiveness (Patton, Stanford & Barratt 1995). Patton and his colleagues added attentional impulsiveness to their understanding of impulsivity and described impulsiveness as comprising the three factors, attentional, motor, and non-planning impulsiveness. This addition of an attentional impulsivity aspect is also in line with the cognitive theory of executive control functioning.

From a mental health perspective impulsivity is regarded as a central component in a wide range of pathological behaviors and disorders, such as ADHD, drug abuse, borderline and antisocial personality disorders, suicidal tendencies, and mania (American Psychiatric Association, 2000). Extensive research on underlying mechanisms has associated serotonin with impulsive behavior (Soubrié 1986, as cited in Evenden, 1999; Evenden, 1999; Fairbanks, Melega, Jorgensen, Kaplan, & McGuire, 2001).

THEORETICAL AND EMPIRICAL BACKGROUND

Measures of Impulsivity: Psychometric and Cognitive Behavioral Assessment

Impulsivity is an extensive term which can be described or defined by a wide variety of different behaviors. Within scientific research on personality and psychopathology several questionnaires and other instruments have been developed for the assessment of impulsivity. The most common method for assessing impulsivity stems from the psychometric personality assessment tradition. Here the subject evaluates him- or herself on a selection of statements about personal preferences and habits, which the researchers believe are related to impulsivity. Examples of such questionnaires are the Barratt Impulsiveness Scale (BIS; Patton et al., 1995), the Dickman Impulsivity Inventory (Dickman, 1990), and the I_7 (Eysenck, 1993). Also, many broader personality inventories include a subscale describing impulsivity, like the Impulsiveness facet from the NEO-PI (McCrae & Costa, 1997). Lane, Cherek, Rhoades, Pietras, and Tcheremissine (2003) examined the relationship between four commonly used psychometric tests of impulsivity (BIS-11, I_7, Dickman Impulsivity Inventory, and Wender Utah Rating Scale). They found correlation between these tests to be consistently high, and all four psychometric tests loaded onto a single factor.

Impulsivity is a broad concept but although psychometric instruments provide good measures to separate more or less impulsive individuals, they do not provide much insight into how impulsivity influences cognitive processes. Experimental behavioral tests are typically more narrowly defined and often developed within a framework of cognitive or neurocognitive theories. Cognitive behavioral tests commonly used to measure impulsivity have been found to divide into several factors.

Swann, Bjork, Moeller, and Dougherty (2002) examined two models of impulsivity, the reward-delay model and the rapid-response model. The tasks assumed to measure reward-delay impulsivity did not correlate significantly with tasks assumed to measure rapid-response impulsivity. Rapid-response impulsivity was associated with lifetime axis I and axis II diagnoses and was more strongly correlated and also contributed significantly to variance in BIS scores. This pattern was replicated by Dougherty et al. (2003) who found that compared with reward-delay tasks, rapid-response tasks were more sensitive to between group differences in impulsivity, in comparison between clinical and control groups. Lane et al. (2003) also found behavioral measures to load into two factors, but not exactly the same as the ones found by Swann et al. and Dougherty et al. They found the response inhibition tasks and the delay of reward tasks to divide into separate factors.

Reynolds, Ortengren, Richards, and de Wit (2006) examined the correlations between three personality measures (BIS-11, I_7, and Multidimensional Personality Questionnaire), and four laboratory-task measures of impulsive behavior, in a healthy population. They found that although correlations between various self-report measures of impulsivity were high, self-report measures were not correlated with behavioral-task measures. They also found that data from behavioral-tasks measures formed two components, impulsive disinhibition and impulsive decision making. Reynolds et al. suggested that self-report and behavioral measures probably measure different constructs, and also that among the behavioral measures, different tasks measure different components of impulsive behavior. This was supported by Lane et al. who found correlations between the behavioral tasks and psychometric instruments to be uniformly low. This illustrates the complexity of the phenomenon impulsivity and poses a challenge when choosing good behavioral measures of impulsivity.

Impulsivity within a Cognitive Framework

From a cognitive perspective, impulsivity can be conceptualized as an issue of controlling thought and action, more specifically, a lack of inhibitory efficiency (Logan & Cowan, 1984). Inhibition is widely accepted as a crucial part of executive functions. A central question in understanding executive functions is whether they are best classified as one unity or as several components (Jurado & Rosselli, 2007). Miyake and colleagues (2000) tried to resolve this issue and found evidence in support of both the unity and the diversity theory. They found three moderately correlated, but clearly separable executive aspects: shifting, updating and inhibition. The moderate correlation indicates a relationship between these aspects. One of their explanations of the common, underlying factor is that all the three targeted executive functions depend on one global inhibitory process.

Greene, Braet, Johnson, & Bellgrove (2008) presented a somewhat different description of executive functions, entailing working memory, response inhibition, and sustained attention. In this description, inhibitory function is involved in at least two of the components. Response inhibition is the suppression of actions that are no longer required or are inappropriate (Logan, 1994). Inhibition is also highly relevant to attention, which relies on the ability to depress disrupting effects of non-pertinent stimuli (Baddeley, 1996).

Executive functions operate on other cognitive functions (Friedman et al., 2008), and are therefore of primary importance in understanding human cognition. The role of impulsivity in a variety of mental and behavioral disorders, including attention deficit and

hyperactivity disorder (ADHD), borderline and antisocial personality disorders, suicidal tendencies, mania, and substance abuse (American Psychiatric Association, 2000) underlines the importance of an understanding of the cognitive processes involved in impulsivity, as well as in the etiology of maladaptive impulsivity.

Response inhibition

Different aspects and definitions of inhibition, such as response inhibition and attentional inhibition, pose the question whether the best theoretical model comprises only one or rather several inhibitory mechanisms. Based on empirical studies of response inhibition, a range of motor actions, including eye movements, hand movements, key presses, squeezes, and speech, can all be stopped very quickly and in approximately the same time (200 ms. for young adults). Logan argues that a single, amodal, central process is responsible for stopping all these actions (Logan, 1994; Logan & Cowan, 1984).

In a study of response inhibition De Jong, Coles, Logan and Gratton (1990) included measurements of neural activity by EEG and obtained indications of two separate stopping mechanisms. Their data suggested that if the central cognitive processes underlying response are completed before the central inhibitory processes can interfere, the overt (motor) response can still be stopped by inhibition of motor commands from central to peripheral structures (De Jong et al.). De Jong and colleagues suggested that the inhibitory mechanism involved in stopping motor responses is faster than the central executive inhibitory mechanism. The central executive inhibitory mechanism is capable of highly specific inhibition, as opposed to a more general and nonspecific inhibition involved in stopping motor responses.

The suggestion of two separate inhibitory mechanisms has later been supported by Aron and Verbruggen (2008) who attempted to tap each mechanism specifically. Subjects were instructed to respond simultaneously with both hands when cued, and to inhibit response from either the right or the left hand occasionally. Aron and Verbruggen's hypothesis was that if response inhibition can be performed in either a slow/specific or a fast/nonspecific fashion, the researchers can facilitate activation of one or the other by manipulating the subjects' knowledge about which response to inhibit. If subjects do not know beforehand which response to inhibit, a general inhibitory mechanism should be activated. If, however, subjects are preinformed which of the two responses to inhibit *if* any inhibition is required, then they have the necessary information to make specific, as opposed to general inhibition. The results supported the hypothesis. When preinformed, the subjects stopped slower, but conducted the non-inhibited response faster.

The relatively slower processing of selective inhibition was also found by Coxon, Stinear and Byblow (2007), but in this study inhibition was measured differently. Instead of inhibiting a response that was cognitively preprogrammed, the subjects were instructed to press and hold two buttons at the presentation of a visual cue. Following another visually presented indicator, the subjects were instructed to release one or both buttons. While the subjects in Aron and Verbruggen's study needed to inhibit the completion of a cognitively preprogrammed response, the subjects in Coxon et al.'s study had to inhibit an action which was already being actively and overtly performed, making the speed of the inhibition more easily observable. Accordingly, Coxon et al. were able to register the inhibition response time simply as the time from onset of the stop signal to the subject's release of the corresponding button. The shortcoming of this method is that it does not separate cognitive from peripheral nervous and muscular processes, so the reaction time obtained is a composite of cognitive and non-cognitive processes.

The race model of inhibition

From a cognitive perspective, an ordinary motor response can be said to be a process of programming and execution. Although we are not able to observe the cognitive process itself, the result of this process can be seen as overt behavior. Response inhibition however, is a process which purpose is to stop another process. Neither of the cognitive processes are directly observable. Because of this, researching inhibitory processes has an inherent problem. While measuring simple go reaction time is a fairly easy and straightforward procedure, measuring the stop reaction time is rather the opposite. Measuring efficiency of inhibition implies measuring the time it takes for a subject to realize that he or she should now withhold action. The result of a successful inhibition is absence of an overt response, but an absent response does not tell the researcher how long it took the subject to process and execute the instruction "do not respond". To assess the efficiency of inhibitory control, it is necessary to find a way to estimate more precisely the time needed to inhibit a response. As previously described, Coxon et al. (2007) omitted this problem by making the subjects inhibit an ongoing overt action, at the cost of not being able to separate the time measurement of cognitive inhibition from the peripheral neural and muscular execution. The race model of inhibition provides a theoretical framework for calculating the time it takes to complete the cognitive inhibitory process without overt behavioral change.

Logan (1994) depicts the relation between the go and stop processes as a race (originally a horse race: Logan & Cowan, 1984). The two processes are assumed to be

separate and independent of each other, one generating the primary response and the other generating the stop function. The purpose of the race analogy is to visualize the concept of two competitors racing toward a finishing line where the first to finish is the winner. If the go process wins the race (finishes first), action is executed. Contrarily, if the stop process finishes first, action processing is inhibited and no action is executed. From this model it follows that the relative speed and the relative starting point of the processes determine the outcome of the race. That is, if the stop process starts after the go process, the stop process can still win the race if it runs sufficiently faster than the go process.

The assumption of go and stop as two separate processes was empirically investigated by De Jong, et al. (1990) who combined a choice reaction/inhibition task with EEG registration of participants. The EEG data showed independent activation by go and stop stimuli. Activity associated with go signals alone was clearly recognizable and distinguishable from activity associated with stop signals. When the stop process was initiated but failed to complete (i.e., inhibition failed and motor response was executed), the go-related EEG activity was not observably different from go-activity when stop-activity was absent. These results can be interpreted as indicating that the two processes are truly parallel and separate and do not influence each other until completion (Aron & Verbruggen, 2008), thereby supporting the fundamental assumption of the race model of inhibition.

Impulsivity as cognitive response style

From the description of the race model it is fair to say that stop signal reaction time constitutes a measure of proficiency with which we can range individuals according to who are better and who are worse at performing the task of response inhibition. The concept it intends to investigate is inhibitory speed and efficiency, and the task is designed to make the inhibition itself a challenge. However, in the stop signal task there is never a question about whether one should inhibit or respond in a given trial, because the go and the stop stimuli are very simple and easily perceptible. This simplicity is rare in the real world, where people typically have to process complex stimuli and make rapid decisions based on imperfect information about existing conditions and possible outcomes.

Personality type ratings of impulsivity typically focus on people's ability and willingness to decide and act rapidly, as opposed to preference for careful consideration, planning and security (Dickman, 1993; Patton et al., 1995). Such strategies tend to be relatively stable in individuals, while the effectiveness and success of each strategy depends greatly on the situation. Accordingly, individuals can be said to vary along a dimension from

impulsive to nonimpulsive, but we can not, on a general level, convert this into a normative scale, saying that individuals at one end perform better than individuals at the other end. It is a matter of individual preference and response *style*, which should not be confused with the *ability* to inhibit a response as discussed in the race model.

Although typically assessed with questionnaires relying on subjects' self evaluation, response style can also be measured by objective behavioral measures, such as the continuous performance test (CPT) (Cornblatt, Risch, Faris, Friedman, & Erlenmeyer-Kimling, 1988). The continuous performance test was originally developed by Mackworth and Taylor (1963) to examine the performance of radar operators. This test requires the subject to pay attention to stimuli presented on a computer screen and respond whenever a target stimulus is presented. Not long after its presentation, researchers started using the test on psychiatric populations in attempts to discover possible neuropsychological deficits (Grunebaum, Weiss, Gallant & Cohler, 1974). Because attentional control is a requirement of this test, it has been frequently used in the assessment of ADHD.

Traditionally proportion of correct responses has been seen as a measure of vigilance (Cornblatt, et al. 1988). Errors of omission, when subjects fail to respond to target, has been interpreted as an indication of failure in vigilance or sustained attention (Halperin, Wolf, Greenblatt, & Young, 1991). Several different versions of CPT have been developed. A common feature is that target is defined by two consecutive stimuli (e.g. the same nonsense figure presented twice, or the letter X following the letter A).

In the Continuous Performance Test - Identical Pairs (CPT-IP) (Cornblatt et al., 1988), a quick decision is required of whether a presented stimulus is identical or not to the last presented stimulus. The essential challenge is to make a quick decision (respond or not respond) even when processing of stimuli is incomplete and one is uncertain whether the stimulus is a target or not. We can think of this decision process in two steps, the first involves comparing the present stimulus with the former. The second step is to handle the uncertainty resulting from incomplete processing of stimuli. When discrimination is less than perfect, one can choose to improve hit rate by increasing response rate, risking more false alarms. Conversely, fewer false alarms can be attained by reducing response rate, at the cost of fewer correct hits. Just as individuals differ in ability to discriminate stimuli, they also differ in how they balance the alternatives of responding or not responding, weighing correct responses against errors. The individual preference can be referred to as response style.

Impulsivity and the Serotonin System

Impulsivity was first linked to central serotonergic function over 30 years ago (Walderhaug, 2007). Through several studies Soubrié (1986, as cited in Evenden, 1999) found a connection between serotonergic neurons and behavioral inhibition, and the association of impulsive behavior with a low capacity of the serotonergic system has been demonstrated in both rodents (Evenden, 1999) and primates (Fairbanks, et al. 2001). It has been established that the serotonin system is somehow involved in impulsivity, however the influence of serotonin in impulsive behavior is only partially understood. Insight into the human genome raised optimism regarding the possibility of finding genotypes underlying mental disorders, but has been followed by awareness of the difficulty in associating genotypes to diagnostic definitions of disorders.

The endophenotype concept

Search for candidate genes for mental and behavioral disorders through the last two decades has revealed a need to understand the mediating neurological and cognitive processes. Endophenotype is a general term for intermediate phenotypes that form causal links between genes and overt expression of disorders. Endophenotypes link observable syndromes that constitute diagnostic categories of disorders to lower level biological processes, thus providing insight into which mechanism may be dysfunctional for a given disorder (Cannon & Keller, 2006).

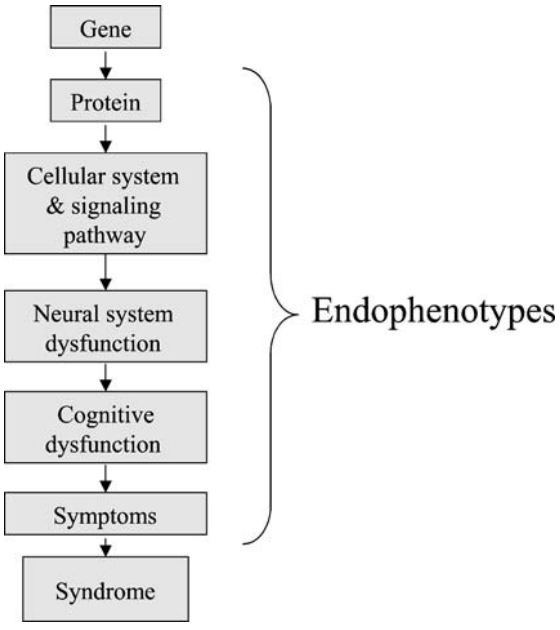

Figure 1. A general description of the connection between genotype, phenotype, and intermediate endophenotypes. Adapted from Cannon and Keller, 2006.

An endophenotype may be neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive, or neuropsychological in nature, and is likely to be less complex than the phenotype disorder (Gottesman & Gould, 2003). Cannon and Keller describe the association between genotype and diagnostic phenotype (Figure 1), illustrating a (simplified) path from gene to syndrome.

The serotonin transporter protein, 5-HTTLPR

Research on impulsivity and the serotonin (5-HT) system has focused on a polymorphism in the promoter region of the gene coding for the serotonin transporter protein.

The process of 5-HT reuptake into the presynaptic neuron, and thereby maintenance of the amount of 5-HT available for subsequent release, is performed by a single protein. This protein, known as the 5-HT transporter (5-HTT), removes 5-HT from the synaptic cleft and determines the magnitude and duration of postsynaptic receptor-mediated signaling, thus playing a pivotal role in the fine-tuning of 5-HT neurotransmission (Lesch & Gutknecht, 2005; Lesch & Mössner, 1998). In other words the 5-HT transporter protein is a crucial element in the 5-HT system, and anything that changes the amount of available 5-HT transporter or alters its functional properties might in theory make significant impact on central nervous processes and subsequently on a subject's behavior. A familiar example of imposed alteration of functional property is selective serotonin reuptake inhibitor drugs (SSRIs) that block the 5-HT transporter and thereby prevents the transporter protein from effecting reuptake. The presence of 5-HT in the synapse is thus prolonged.

In 1996, a newly discovered common variation in the promoter region of the 5-HTT gene was discovered, a biallelic polymorphism comprising a long (L) and a short (S) variant (Heils et al.1996; Lesch et al.1996). This polymorphism is now commonly referred to as the serotonin transporter protein gene-linked polymorphic region, or the 5-HTTLPR. The promoter region of a gene is responsible for regulating transcription (mRNA synthesis) of that gene, subsequently leading to protein synthesis.

Using in vitro studies of lymphoblast cells[1] to investigate a potential functionality of the long/short polymorphism, Heils and colleagues found that the L variant was associated

[1] Lymphoblasts are cells of the immune system that express functional 5-HT and possess an uptake system based on the 5-HT transporter protein (Faraj, Olkowski, & Jackson, 1994). Lymphoblast cells are not involved in central nervous 5-HT systems, but are more easily available than central nervous 5-HT cells, which make lymphoblasts eligible for research.

with approximately three times higher transcriptional activity compared to the S variant. Lesch et al. supported this finding and also showed that the higher activity of the L allele is most effective in cells homozygous for the L allele, as opposed to heterozygous SL cells whose transcriptional activity more closely resembles that of homozygous SS cells. The rate of 5-HT uptake was double in LL cells compared SL or SS cells. The data for SS and SL were similar on both mRNA concentrations and 5-HT uptake, and these genotypes differed from LL genotype, indicating a functional dominance of the S allele over the L allele.

Later this association has been clouded by Hranilovic et al. (2004) who found a similar but non-significant trend in mRNA expression, and by Lim, Papp, Pinsonneault, Sadée and Saffen (2006) who found no association. Further complicating the picture, Nakamura, Ueno, Sano and Tanabe (2000) identified several new varieties in the DNA sequence, revealing that the 5-HTTLPR can be found in at least 14 variants, and that the two alleles S and L are actually four and six different allelic variants, respectively.

A more recent study concluded that three alleles are commonly found in North European, Caucasian American, African American and Indian American populations (Hu et al., 2006). These three allelic variants are S, L_G and L_A. The long alleles, L_G and L_A are distinguished by an A→G single nucleotide polymorphism (SNP). Hu et al. found L_G to be nearly equivalent to S regarding mRNA expression. Following this they argued that L_G should be treated as analogous to S and separate from L_A, when searching to explain neurological or behavioral characteristic by 5-HTTLPR variability. The recent discovery of a functionally significant SNP in the L allele poses a considerable challenge to the interpretation of previous findings as well as to the possibility of comparing results from studies using triallelic as opposed to biallelic classifications. The lack of differentiation between L_G and L_A is a possible explanation for inconsistencies in results across studies.

Studies relying on PET methodology have found increased 5-HTT availability and increased 5-HTT binding potential in LL and L_AL_A genotypes, respectively (Heinz et al., 2000; Praschak-Rieder et al., 2007). However, two PET studies reported no significant difference in 5-HTT binding potential (Shioe et al., 2003; Parsey et al., 2006). Additionally, two SPECT studies reported no significant effect of genotype on 5-HTT binding in a range of sub cortical brain structures (Jacobsen et al., 2000; Willeit et al., 2001). Another SPECT study indicated a complex relation, reporting significantly greater 5-HTT availability in SS subjects compared to SL, a non-significant tendency of increased availability in LL compared to SL ($p=0.09$), and no difference in availability between SS and LL (van Dyck et al., 2004). Divergent results have also been found in studies of platelet 5-HT uptake (Nobile et al., 1999;

Preuss et al., 2000; Stoltenberg et al., 2002) and in studies of 5-HTT expression and binding potential in post-mortem brain tissue (Du et al., 2000; Lim et al., 2006; Little et al., 1998). Moreover, the repeatedly reported dominance of the S allele was challenged by Hu, Zhu, Lipsky and Goldman (2004) who found co-dominant effects in a lymphoblast cell study, and by Nobile et al. (1999) who reported 35% higher 5-HT uptake rate in SL compared to SS group.

The combined results from in vivo studies are inconclusive about the effect of 5-HTTLPR on 5-HTT function. Results are divergent within each methodological approach, suggesting that more research is needed before any conclusions can be drawn.

It is possible that 5-HTTLPR has a smaller effect on the adult central nervous system than on earlier development. Neurotransmitters such as 5-HT appear to be endogenous growth signals regulating morphogenetic activities during early embryonic development, including cell proliferation, migration and differentiation (Lauder, 1993). Evidence suggest that allelic variation in functional 5-HTT expression plays a crucial role in synaptic plasticity, thus setting the stage for expression of complex traits and their associated behavior throughout adult life (Lesch & Mössner, 1998).

Lack of in vivo effect of 5-HTTLPR has led some researchers to conclude that associations of the 5-HTTLPR polymorphism to clinical phenotypes appear to be due to developmental effects of 5-HTTLPR on expression rather than to its direct effect on serotonin transporter binding (Parsey et al., 2006). Even though studies of 5-HTT expression and 5-HT reuptake are ambiguous, an effect of the polymorphism on behavior may still be detected due to developmental mediation.

5-HTTLPR and impulsive personality

Several correlation studies have investigated the association between 5-HTTLPR and impulsive personality. Lesch et al. (1996) found a significant correlation between 5-HTTLPR and the NEO-PI facet Impulsivity in a primarily male population, where subjects with one or two S alleles tended to have more impulsive personality than subjects homozygote for L allele. A similar, but non-significant trend was found in a primarily female population (Greenberg et al. 2000). 5-HTTLPR S allele has also been associated with increased disinhibiton (Paaver, Kurrikoff, Nordquist, Oreland, & Harro, 2008; Aluja, Garcia, Blanch, De Lorenzo, & Fibla, 2009).

Sakado and colleagues (Sakado, Sakado, Muratake, Mundt, & Someya 2003) assessed genotype and impulsivity, measured by BIS, in a nonclinical, male Japanese population.

Because of a very low frequency of LL genotype in their sample (as is typical of East-Asian populations; Katsuragi et al. 1999; Nakamura, et al. 2000), Sakado et al. (2003) collapsed LL and LS into one group. They found significantly higher impulsivity scores in the SS group compared to the L carriers. However, in a healthy male Korean population, Lee, Kim and Hyun (2003) separated high, low, and intermediate impulsive individuals by BIS scores and compared the high scorers with the low scorers. They found no significant differences in genotype frequency in the two groups, but allelic frequencies were significantly different. The most impulsive subjects possessed relatively fewer S alleles and more L alleles than the least impulsive subjects. That is, in Lee et al.'s sample, increased impulsivity was associated with fewer S alleles and more L alleles, which is opposite of what one would expect based on the studies cited above.

Although only reporting data for the broad trait neuroticism, Sirota, Greenberg, Murphy and Hamer (1999) results are interesting because the reported association between 5-HTTLPR genotype and personality trait was nonlinear. Subjects with extreme neuroticism scores were selected for genotyping based on the hypothesis of high frequency of S alleles in subjects high on neuroticism, and low frequency of S alleles in subjects low on neuroticism. Results of the genotype-neuroticism association showed that in the high-neuroticism group, S allele frequencies increased with neuroticism only to a certain point. Then it decreased and approximated the frequency for the population with neuroticism scores within normal range. Likewise, in the low-neuroticism group, S allele frequency decreased with neuroticism only to a certain point, and then increased to approximately the level of the normal-neuroticism population. The subjects with the most extreme neuroticism scores did not fit the predicted pattern and deviated strongly from the linear trend that was observed between S alleles and the less extreme scores of neuroticism. This finding, if replicable in relation to impulsivity, is potentially important for two reasons. First, a simple analysis of variance, which is typically applied when the relation between two variables is expected to be linear, is poorly suited for handling nonlinear relationships. A potent, nonlinear trend might thus appear weak or nonexistent. Second, clinical populations are often characterized by extreme personality scores, and clinical significant impulsivity may not be associated with 5-HTTLPR in the same way as impulsivity in normal populations.

None of the cited personality studies investigated the SNP in the L allele, thus separating the L_A and L_G alleles may alter results or explain diverging findings.

5-HTTLPR and pathological impulsivity

Impaired impulse control is included as a general diagnostic criterion for personality disorders, and impulsivity is associated with borderline and antisocial personality disorders in particular. 5-HTTLPR short allele has been associated with borderline and antisocial personality disorder. Incidence of BPD and APD traits was significantly increased for subjects with SS genotype compared to LL subjects, while the difference between LL and SL subjects was not significant (Lyons-Ruth et al. 2007).

Jacob et al. (2004) compared healthy control subjects with patients with personality disorders within clusters B and C, and found no difference in 5-HTTLPR genotype distributions between any of the three groups. Later several studies have replicated this with regard to borderline personality disorder in particular (Ni et al. 2006; Pascual et al. 2008; Tadić et al. 2009). In summary, subjects who fulfill diagnostic criteria for BPD show no association with 5-HTTLPR. The findings of Lyons-Ruth et al. appear to contrast this.

However, an important difference between these studies is that while all the studies reporting negative findings simply categorized subjects according to fulfilled as opposed to not fulfilled diagnostic criteria, Lyons-Ruth et al. investigated the association between genotype and number of pathological personality traits, as defined in DSM-IV (American Psychiatric Association, 2000). This difference has at least two implications.

First, it is unlikely that a single, common genetic polymorphism directly causes or strongly influences a complex phenotype such as a personality disorder. The difference in allelic frequency between a population which reaches diagnostic threshold, and a population of all subjects who do not reach diagnostic threshold, should be very small. Second, measuring borderline and antisocial personality traits should have increased variance of personality and made the study of Lyons-Ruth et al. more sensitive to detect an association than the studies categorizing subjects according to diagnosis.

Attention-deficit hyperactivity disorder is highly associated with impulsivity, and deficits in both inhibitory motor control and attentional inhibition are regarded central and defining components of this disorder (American Psychiatric Association, 2000; Barkley, 1999). Several studies have found that the L allele is associated with ADHD symptoms and diagnosis (Kent et al., 2002; Manor et al., 2001; Retz, Thome, Blocher, Baader, & Rössler, 2002; Retz et al., 2008), but a large study found no association between 5-HTTLPR and ADHD (Xu et al. 2008).

One could argue that ADHD is a category too broad to be used as an operational definition of impulsivity. Hyperkinetic disorder (HD) (World Health Organization, 1993) is a

disorder with a high degree of conceptual similarity with ADHD, but defined by a stricter set of diagnostic criteria, essentially requiring a higher degree of impairment. Seeger, Schloss and Schmidt (2001) found that, compared to controls, patients with hyperkinetic disorders and patients with HD and comorbid conduct disorder showed an enhanced expression of the LL genotype. Unfortunately, few studies have investigated 5-HTTLPR in hyperkinetic disorder.

Overall then, the results are mixed regarding 5-HTTLPR in ADHD and HD, but the main trend in published studies seem to be that ADHD and HD are associated with the L allele.

Associating a specific allelic variation at the genetic level with a given syndrome is difficult because of the complexity of syndromes. Most syndromes are the phenotypic result of multiple genotypes and multiple intermediate levels that affect each other and are affected by other biochemical and environmental influences, making the explained variance from each gene very small. Endophenotypes are conceptually simpler and etiologically closer to the genotype, which makes it easier to find the genes associated with them.

A simple and straightforward way to apply the concept of inhibition to an understanding of dysfunctional impulsivity is to say that people who are not impulsive are relatively good at inhibiting action, while impulsive individuals have trouble inhibiting action. The stop signal task has been found to differentiate pathologically impulsive from healthy populations. Response inhibition has been proposed as a candidate endophenotype for a variety of disorders related to dysfunctional impulsivity including ADHD (Aron & Poldrack, 2005; Schachar, et al., 2005; Slaats-Willemse, Swaab-Barneveld, de Sonneville, van der Muelen, & Buitelaar, 2003), obsessive-compulsive disorder (Chamberlain et al., 2007; Menzies et al., 2007), psychopathic personality (Newman, Widom, & Nathan, 1985), pathological gambling and alcohol dependence (Goudriaan, Oosterlaan, de Beurs, & van den Brink, 2006). Extended knowledge about the genes affecting response inhibition can thus prove valuable in the understanding and treatment of disorders associated with deficient response inhibition.

Are effects of 5-HTTLPR variability different in men and women?

On the basis of observed differences in incidence of a variety of psychological disorders among men and women it has been speculated that men and women respond differently to variation in 5-HT function. Examples of disorders that occur unevenly among male and female populations are depression, ADHD, borderline personality disorder, and

antisocial personality disorder. Men show a higher prevalence of impulsive control disorders, substance use and antisocial personality disorder (Holden, 2005; Kessler et al., 2005). It has also been found that men and women respond differently to serotonin deficiency that is experimentally induced by tryptophan depletion. A review of tryptophan depletion studies suggested that females are generally more vulnerable to the reduced serotonin availability that occurs from tryptophan depletion (Bell, Hood, & Nutt, 2005). A recent study found that tryptophan depletion significantly affected mood in women but not in men. Men employed a more impulsive response style when depleted, while women became more cautious (Walderhaug et al., 2007). Assuming that 5-HTTLPR variability contribute to variation in the 5-HT system, it follows that sex should be included as a variable in studies of 5-HTTLPR and behavior.

A majority of studies on 5-HTTLPR and impulsivity have either only studied one of the sexes, or have mixed men and women without attending to the possible effects of this variable. Thus, these studies are not able to reveal potential effects of sex, and their results might be confounded by dissimilar effects between sexes. Other studies have found that 5-HTTLPR interacts with sex in relation to psychometrically assessed impulsivity (Paaver et al., 2008), suicide attempts (Baca-Garcia et al., 2002), and vulnerability to development of depression (Sjöberg et al., 2006). These results suggest a sex difference in how 5-HTTLPR contributes to behavioral phenotypes (Baca-Garcia et al., 2002). To avoid confounding and to strengthen validity of the present study we will consider the possible mediation by sex on the association between 5-HTTLPR and impulsivity.

5-HTTLPR and objective behavioral assessments of impulsivity

To our knowledge only three studies have investigated the association between 5-HTTLPR and objective behavioral measures of impulsivity. Paaver et al. (2007) found that the S allele was significantly associated with more impulsive response style and higher error rate on a visual comparison task in a sample of nearly four hundred adolescents. Clark et al. (2005) found no association between 5-HTTLPR and stop signal reaction time on the SST, nor with sensitivity to tryptophan depletion. Walderhaug et al. (2007) found no main effect of 5-HTTLPR on response style as indicated by the β measure from CPT-IP. However, men and women responded differently to tryptophan depletion. While there was no significant difference between men and women in the placebo control condition, men became more impulsive and women became more cautious during depletion, supporting the assumption that impaired 5-HT function affects men and women differently.

As is typical of the reviewed literature on 5-HTTLPR and various aspects of impulsivity, findings are mixed. However, very few studies have investigated the effect of 5-HTTLPR on objective behavioral measures of impulsivity, and more research is needed.

Purpose of the Study

1. The primary aim of this study is to investigate the association between 5-HTTLPR and impulsivity in healthy adults with the genotyping procedure respecting the functional SNP in the L allele.
2. A subordinate purpose of the study is to investigate possible sex dependent effects of 5-HTTLPR variation on impulsivity.

METHOD

Participants

The data analyzed in this report were collected as part of the project "Cognitive control, mood, brain function and genetics in major depressive disorder and healthy people" (http://humancognition.org). The principal investigator of this project is Nils Inge Landrø. The project was approved by the Regional committee for research ethics. Participants for this project were recruited in several ways. Some were recruited by newspaper advertisement. Others were acquaintances of the researchers working on this project, such as friends or family members.

Psychiatric Evaluation

Medical history was recorded for all participants. A structured interview screening for axis I disorders and the SCID I module for affective disorders were administered to all subjects. Depending on screening results, other SCID I modules were administered. For all subjects included in the analysis the administration of screening and SCID I was audiotaped. Cases that were difficult to diagnose were discussed in a group with other testers and a diagnostic expert. In addition, a few random cases were selected and audiotapes reviewed in the research group to ensure diagnostic reliability. Personality disorders were assessed by SCID II.

From an overall database of nearly two hundred subjects, all subjects who met criteria for any axis I or axis II disorder (current or previous) or ADHD were excluded. Subjects who reported current or previous alcohol or drug abuse were also excluded. After excluding additionally two subjects who reported head injuries that had led to more than thirty minutes uncounsciousness, our remaining dataset consisted of 87 healthy subjects.

Stop Signal Task

Stop signal tests is a widely used paradigm for measuring inhibitory control (Aron, Dowson, Sahakian & Robbins, 2003a; Clark, et al. 2005; Logan & Cowan, 1984; Logan, Cowan & Davis, 1984; Logan, Schachar & Tannock, 1997). We used the Stop Signal Task (SST) from the CANTABeclipse^{TM} software battery of neuropsychological tests (Cambridge Cognition Limited, 2006). The stop signal task is based on the race model depicting controlled action programming/execution (go) and inhibition (stop) as separate cognitive processes. In this paradigm participants are programmed to a choice reaction to a stimulus, and inhibitory control is defined as the subject's ability to inhibit the prepotent motor response.

SST consisted of two parts. In the first part, the subjects were introduced to a two-button press pad and told to press the left hand button when they saw a left-pointing arrow, and the right hand button when they saw a right-pointing arrow. There was one initial block of 16 trials, which was for practice and introductory purpose only and was excluded from analysis. In the second part, the subjects were told to continue pressing the buttons on the press pad when they saw the arrows, as before, but if they heard an auditory signal (a beep), they should withhold their response and not press the button. They were informed that they would not always be able to stop, but sometimes they would, and were encouraged to try their best. This part consisted of five blocks, each containing 64 trials, of which 16 (25%) were "stop" trials. Between each block the computer supplied graphic and written feedback on the subject's performance. Subjects who responded relatively slow were encouraged to try to respond faster, while subjects with low inhibition success rate were reminded to inhibit response when the beep appeared. The experimenter repeatedly informed all subjects that fast response and inhibition were both equally important.

The test provides the known variables go signal reaction time (GoRT) and stop signal delay (SSD). All else being equal, GoRT is an indicator of the subject's psychomotor speed, reported as the time (milliseconds) between onset of go stimulus and registered correct response. SSD is the delay between the stimulus onsets of go signal and stop signal. This delay is manipulated experimentally (by the computer software), with the implication of making inhibition easier (if delay is decreased) or more difficult (if delay is increased).

Several ways of calculating stop signal reaction time have been proposed (Logan, Cowan & Davis, 1984; Logan, et al. 1997; Osman, Kornblum & Meyer, 1986). The easiest, and probably most accurate, is the method developed by Osman et al. (1986) and refined by Logan et al. (1997). This is based on a tracking procedure in which SSD changes after every stop signal trial, increasing by 50 ms if subjects inhibit and decreasing by 50 ms if they fail to inhibit. The tracking converges SSD on a delay where each subject successfully inhibited on 50% of the stop trials. Based on this, three assumptions are made: 1) the race is now "won" 50% of the times by the go and stop processes respectively, 2) at this particular delay the go- and the stop processes finishes at the same time, 3) which process happens to win on a given trial depends on random variation (Logan et al., 1997). Because subjects inhibit 50% of the time, mean GoRT must equal SSD pluss SSRT.

Go-signal reaction time	=	Stop signal delay	+	Stop signal reaction time
(GoRT)		(SSD)		(SSRT)

In stop-signal paradigms relying on this calculation, GoRT is obtained from simple go trials and SSD is manipulated by the researcher, and so the values of these variables are known. Subsequently SSRT is calculated by subtracting SSD from GoRT (Logan et al., 1997).

$$GoRT - SSD = SSRT$$

The advantage of this method is that it isolates inhibition processing time from noncongitive processes.

The Continuous Performance Test - Identical Pairs

The continuous performance test - identical pairs (CPT-IP) (Cornblatt et al., 1988) required the subjects to identify identical stimulus pairs within a continuously presented series of stimuli. Subjects were instructed to respond as quickly as possible whenever two identical stimuli were presented in a row. The subjects responded by lifting their finger from the computer mouse held in the dominant hand. Before the test started the test administrator let the participants practice with cards illustrating the principles of the test. In addition there was a practice session before the test started where the participants were presented three digit numbers and required to respond whenever two identical numbers were flashed on the computer screen in a row. The practice session consisted of 25 trials.

The test consisted of one session of four digit numbers and one session of nonsense shapes. The sequence in which the sessions were presented was randomized across subjects. Each stimulus was presented for a duration of 50 ms. at a constant rate of one stimulus per second. Each session consisted of 150 trials out of which thirty in each mode were target trials. These required response, and correct responses were named “hits”. An equal number of catch trials that were similar, but not identical to target stimuli, were also presented in each mode. Response to catch trials is referred to as “false alarm”.

Traditionally, proportion of correct responses (hits) has been considered a measure of vigilance, and missing targets has been interpreted as an indication of failure in vigilance or sustained attention (Cornblatt et al. 1988). Two other types of errors are also possible. Responses to stimuli that are not similar to the target are considered random errors. These errors occur at a very low rate in normal controls. These errors may be explained by diffuse perceptuo-motor dyscontrol (Cornblatt et al., 1988). Random errors have received relatively little attention by researchers. Responses to target-similar stimuli, called false alarms, have

traditionally been interpreted as reflecting impulsivity (Dougherty et al. 1999, 2003; Halperin, et al. 1991). Yet proportion of false alarms is not a pure measure of impulsivity because false responses are likely moderated by the individual's ability to discriminate stimuli.

Signal detection analysis has been used to combine hits and false alarms into measures of attentional capacity and response style, the d' (d-prime) and β (beta) (Swets, Tanner, & Birdsall, 1961; Walderhaug et al. 2002). β is a measure of response bias, essentially weighting the rates of hits and false alarms. Since target and catch trials were equally frequent in the stimulus material, no response bias is indicated by $\beta = 1$. When $\beta < 1.0$ the individual has a risk-taking response tendency that results in overresponding (yielding more hits and more false alarms). $\beta > 1.0$ indicates that the individual has a cautious response style and is biased towards underresponding. Increasingly, the d' and β measures have been considered the best approach for extracting and describing the processes underlying sustained attention and response style (Conners, Epstein, Angold, & Klaric, 2002; Cornblatt et al., 1988; Epstein et al., 2003; Keilp, Herrera, Stritzke, & Cornblatt, 1997; Walderhaug et al., 2007).

5-HTT Genotyping and Classification

A blood sample was drawn from each participant in cooperation with the Psychopharmacological department at Diakonhjemmet Hospital. Blood samples were analyzed at the Clinical Chemical Department at Oslo University Hospital, Ullevål, for genotyping. The procedure for genotyping the triallelic 5-HTTLPR polymorphism located in the SLC6A4 gene, coding the serotonin transporter protein (HTT), was performed essentially as described in detail elsewhere (Gelernter, Kranzler & Cubells, 1997; Stein, Seedat & Gelernter, 2006).

Briefly, genomic DNA was amplified by polymerase chain reaction (PCR) on a real-time fluorescence LightCycler instrument in a final volume of 20 µl using LightCycler Faststart DNA SYBR Green kit (Roche cat no 12239264001) with specific primers (0.5 uM) (Gelernter, Kranzler & Cubells, 1997) generating a long (L) 419 bp or a short (S) 375 bp PCR product depending on the presence of a 16 bp or a 14 bp sequence repeat, respectively, in the promoter region. Cycle conditions were the following: 10 min denaturation (95^{o} C), 45 cycles at 95^{o} C (10 s.), 66^{o} C (10 s.) and 72^{o} C (0 s.). For the detection of the additional A/G single nucleotide polymorphism (SNP) that occurs within the L fragment (L allele), the PCR fragments were digested with 1 U MspI restriction enzyme

(New England Biolabs, Beverly, Massachusetts) for 2 hours at 37^{o} C. The PCR fragments contain two obligatory MspI sites, whereas the A/G substitution creates an additional MspI site. Thus, a single PCR reaction and restriction digest followed by size fractioning on a gel provides classification of the S, L_G and L_A alleles. To ensure reliability of genotyping, extracted DNA and classification from Yale University School of Medicine, used in Walderhaug's (2007) doctoral dissertation, were used as control samples.

Since the discovery of the A/G SNP in the L allele, many studies adhere to the triallelic classification of genotypes described in Table 1 (Neumeister et al., 2006; Ni et al., 2006; Parsey et al., 2006; Praschak-Rieder et al., 2007; Walderhaug et al., 2007). This was the primary mode of genotype organization in our study. Accordingly, when referring to our own data, SS, L_GS, and L_GL_G genotypes are simply called SS. L_AS and L_AL_G genotypes are referred to as SL. L_AL_A genotypes are referred to as LL.

Table 1. Categorization of 5-HTTLPR genotypes according to functional quality, and reclassified codes for easier reporting.

Genotype	**Functional quality**	**Reclassified**
SS, SL_G, and L_GL_G	homozygous low expressive group	SS
SL_A and L_GL_A	heterozygous low/high expressive group	SL
L_AL_A	homozygous high expressive group	LL

With respect to the various ways the 5-HTTLPR genotypes have been organized and analyzed in previous research, data from the SSRT and the CPT β and false alarms was reanalyzed with genotypes classified the "old way", ignorant of the A/G single nucleotide polymorphism. A dominance model where low expressive alleles were considered dominant was also investigated with and without consideration of the SNP. These analyses most often made little difference compared to the primary analyses, so results from the secondary analyses are only reported in detail when they deviate notably from primary analyses.

Statistical Analysis

Statistical analyses were performed using SPSS 16.0 for Windows. Criterion for exclusion of extreme values was set to two standard deviations for dependent variables.

One-way analysis of variance (ANOVA) was used to investigate main effects of genotype. Dependent variable from the stop signal task was SSRT. Proportion of successful

stops (PSS) was used as a control variable to ensure that the combination of instructions and computerized tracking procedure had succeeded in converging on a 50% inhibition success rate. Dependent variables from the CPT-IP are β and false alarm, both potential measures of impulsivity. Possibly loading on different modules (Cornblatt et al. 1988; Walderhaug et al. 2002), the shapes and numbers conditions were analyzed separately. The d' measure of CPT is an indicator of the subjects' ability to discriminate between stimuli. Because a difference in this ability could complicate interpretation of results, between-groups comparisons of d' were also conducted. Univariate ANOVA was performed to investigate possible genotype x sex interaction effects on SSRT, β and false alarms. All alpha levels were set to 0.05. When ANOVA indicated significance, post hoc tests with Bonferroni corrections were performed.

RESULTS

Demographic and Psychometric Characteristics

The sample consisted of 87 subjects. Mean age was 36.4 years (SD = 12.4), ranging from 19 to 61 years. Mean years of education was 16.6 (SD = 2.5) years. Mean estimated verbal intelligence was 10.8 (SD = 3.23), mean estimated nonverbal intelligence 13.4 (SD = 3.04). Median values for Beck´s Depression Inventory and Beck´s Anxiety Inventory were both 1 (BDI SD = 3.9; BAI SD = 3.1).

Genotype Distributions

The genotype frequency distribution within the sample was similar to distributions reported in comparable North-European samples (Hu et al., 2006), thus representativeness of the population was assumed. The genotype distribution was SS 18%, SL_G 6%, L_GL_G, 1%, SL_A 39%, L_GL_A 13%, L_AL_A 23%.

SST

Two subjects exceeded two standard deviations on stop signal reaction time and were excluded from analyses. Mean proportion of successful stops across the remaining 85 subjects was 0.51 (SD = 0.09). This indicated that the task was performed as intended and that the theoretical assumptions, on which the stop signal reaction time was calculated, were met.

Mean reaction time to the stop signal (Figure 2) implied a pattern where participants in the SS group had longer stop signal reaction time (SSRT) than those in the SL group. The participants in the LL group had the shortest SSRT. This implied that L_A allele was associated with better performance on the inhibition task. The same trend seemed to be present for both male and female participants (Table 2).

Levene's test of homogeneity confirmed that groups were not significantly different in variance (p = .267), thus validating use of the F test. A one-way ANOVA was applied to investigate the effect of independent variable genotype on dependent variable SSRT. Results showed that the observed effect was significant (F (2, 82) = 3.264, p = .043). Post hoc tests with Bonferroni corrections revealed that this significance was primarily due to the LL group's faster reaction time compared with the other two groups. Although none of the post hoc comparisons were significant, the LL group approached significance with the SS group

(p = .058), and showed a tendency do differ from SL group (p = .104). The Bonferroni post hoc indicated no difference between SS and SL (p = 1.000).

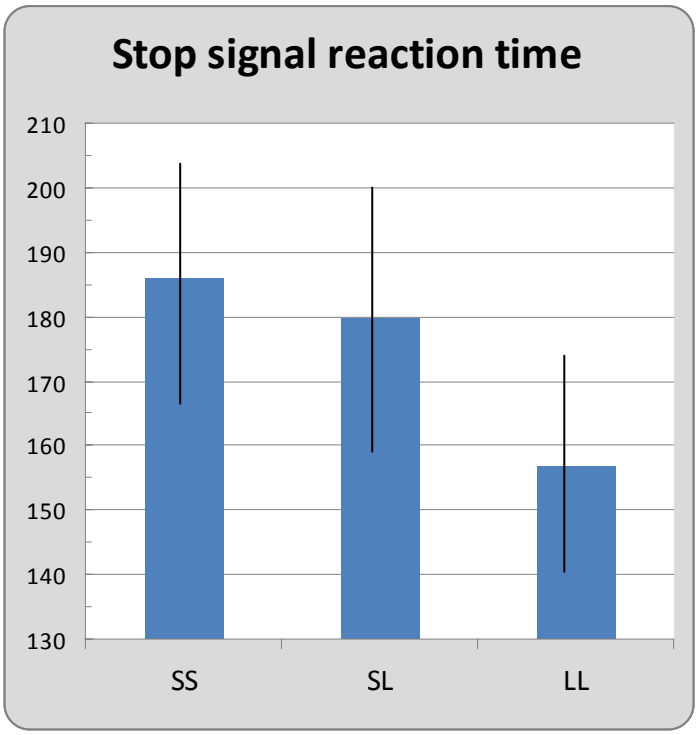

Figure 2: Mean stop signal reaction time for each of the groups according to genotype. Values on the vertical axis are milliseconds; black lines indicate the standard deviations. Genotype descriptions are classified according to the triallelic model.

Mean age of participants was higher in the SS group (42.3 years) than in the SL group (34.8 years) and the LL group (32.9 years). A oneway ANOVA confirmed that the age differences were significant ($F(2,82) = 3.722$, $p = .028$). Therefore a univariate ANCOVA for genotype and SSRT with age as a covariate was conducted. This resulted in a significant effect of age on SSRT ($F(1,81) = 4.482$, $p = .037$), and a non-significant effect of genotype on SSRT ($F(2,81) = 2.373$, $p = .100$).

Stop signal reaction time data were reanalyzed with a different classification of genotypes using ANCOVAs with age as a covariate. When assuming dominance of the S allele and not considering the SNP the effect of genotype approached significance ($F(1,82) = 3.472$, $p = .066$) and the effect of age was significant ($F(1,82) = 5.054$, $p = .027$).

When considering the SNP and assuming a dominance of the S and L_G alleles over L_A, both genotype ($F(1,82) = 4.796$, $p = .031$) and age ($F(1,82) = 4.949$, $p = .029$) significantly predicted stop signal reaction times.

A subordinate aim was to investigate the possible effect of sex on the relationship between genotype and inhibition. Univariate ANOVA with genotype and sex as independent variables showed no effect of sex ($F(1,79) = 0.146$, $p = .703$), and no genotype x sex interaction ($F(2,79) = 0.585$, $p = .560$) on stop signal reaction time.

Table 2: Stop signal reaction times. Means, standard deviations (SD) and number of subjects listed for each group.

Genotype (reclassified)	Total		Male		Female	
	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N
SS	186 (38)	21	188 (39)	7	186 (39)	14
LS	180 (43)	44	175 (52)	16	183 (38)	28
LL	157 (31)	20	167 (31)	8	151 (30)	12
Total	176 (40)	85	176 (44)	31	176 (38)	54

CPT-IP

Data from the CPT-IP were analyzed with one-way and univariate ANOVAs. The dependent variables d', false alarm and β were analyzed from the shapes and numbers modes of the test. CPT data from one subject was missing due to technical problems, and so the total number of subjects available for analysis was 86. The criterion of maximum two standard deviations was applied to each dependent variable, which resulted in the following number of exclusions for shapes and numbers, respectively: 3 and 2 (d'), 1 and 5 (false alarms), 2 and 2 (β). Levene's tests confirmed that after exclusion of extreme scores, the assumption of homogeneity of variances was not violated for any of the analyses performed.

One-way ANOVAs yielded no significant differences between groups on either d' measures (shapes $F(2,80) = 0.601$, $p = .551$; numbers $F(2,81) = 1.549$, $p = .219$). Thus we had no reason to believe that the groups differed on their ability to discriminate stimuli.

False alarms measures

From the means data (Table 3) the difference in proportion of false alarms between groups appeared to be generally small, but slightly higher among the heterozygous individuals compared to SS and LL. The one-way ANOVA showed no significant effect of genotype on false alarms in the shapes ($F(2,82) = 0.904$, $p = 0.409$) or the numbers ($F(2,78) = 1.699$, $p= 0.190$) conditions.

In the reanalysis not considering the SNP, results approached significance on the false alarms numbers condition ($F(2,78) = 3.015$, $p = .055$). With this classification, SL subjects had the largest proportion of false alarms (0.24), followed by LL (0.19) and SS (0.16).

Table 3: β and false alarms. Group means, standard deviations (SD) and number of subjects (N).

Sex	Genotype (reclassified)	β				False alarms			
		shapes		numbers		shapes		numbers	
		Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N
Total	SS	1.61 (0.87)	21	1.08 (0.66)	21	0.16 (.12)	21	0.18 (.11)	20
	LS	1.36 (0.98)	43	0.99 (0.54)	42	0.20 (.12)	43	0.23 (.12)	42
	LL	1.19 (0.73)	20	0.90 (0.41)	21	0.17 (.13)	21	0.19 (.08)	19
	Total	1.39 (0.90)	84	0.99 (0.54)	84	0.18 (.12)	85	0.21 (.11)	81
Male	SS	2.09 (1.01)	6	0.75 (0.54)	6	0.14 (.16)	7	0.23 (.12)	7
	LS	1.35 (0.93)	15	0.90 (0.49)	14	0.19 (.09)	15	0.25 (.15)	15
	LL	1.25 (1.06)	7	1.03 (0.40)	8	0.14 (.13)	8	0.18 (.09)	7
	Total	1.48 (1.00)	28	0.90 (0.47)	28	0.16 (.12)	30	0.23 (.13)	29
Female	SS	1.43 (0.77)	15	1.21 (0.67)	15	0.16 (.11)	14	0.16 (.10)	13
	LS	1.37 (1.03)	28	1.03 (0.57)	28	0.20 (.13)	28	0.22 (.10)	27
	LL	1.16 (0.53)	13	0.83 (0.41)	13	0.19 (.12)	13	0.20 (.08)	12
	Total	1.34 (0.86)	56	1.03 (0.57)	56	0.19 (.12)	55	0.20 (.10)	52

β measures

When visually examining the descriptive data of the β values for both sexes combined (Table 3) we observed a linear trend between the three groups, in the direction of highest β for SS subjects and lowest β for LL subjects. This was evident for both the shapes and the numbers conditions, indicating that LL subjects had a more impulsive response style than SS subjects. The between-groups difference was larger in the shapes condition, but here the standard deviations were also larger. A one-way ANOVA was performed and showed no statistically significant effects for either shapes ($F(2,81) = 1.187$, $p = .310$) or numbers ($F(2,81) = .558$, $p = .574$). The main reason for this may be the high within-group variance reflected by the high SDs.

Although plots indicate an interaction effect of sex and genotype in the numbers condition (Figure 3), this effect was not significant according to univariate ANOVA ($F(2,78)$

= 1.724, p = .185). No interaction effect was found in the shapes condition, (F(2,78) = 0.874, p = 421). All β reanalyses of genotype main effects and of genotype x sex interactions resulted in higher p-values (less significant) compared to primary analysis.

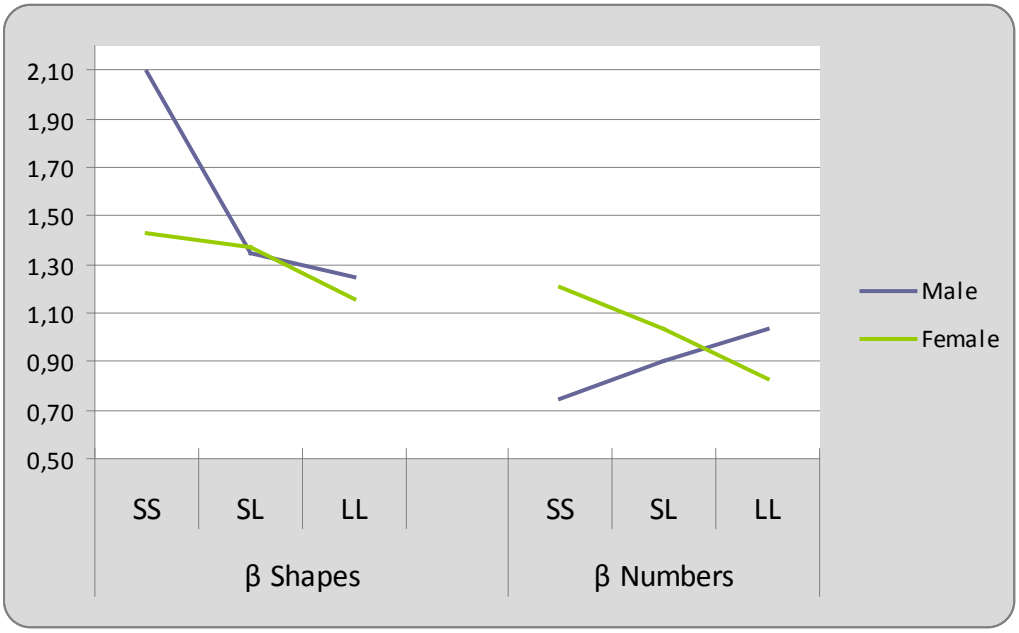

Figure 3: Graphic presentation of β values for each genotype group, classified according to the triallelic model, in the shapes and numbers conditions. Graphs are separated for men and women.

DISCUSSION

The main intention of the study was to investigate the relationship between the serotonin transporter polymorphism and impulsivity in healthy individuals. The available research literature did not allow clear predictions regarding whether an effect of genotype on inhibition and response style should be expected. Very few studies have applied objective laboratory measures to investigate the serotonin transporter polymorphism (5-HTTLPR) in relation to impulsivity. Based on this, we aimed to further examine the association between the serotonin transporter polymorphism and two objective behavioral measures of impulsivity, the stop signal task and the continuous performance test.

Effect of 5-HTTLPR on Inhibition

The primary results showed a significant effect of genotype on inhibition. LL was found to be associated with faster stop signal reaction time in the stop signal task, indicating more efficient inhibition. Subjects carrying the low expressive alleles (S and L_G) were slower to complete the process of inhibiting a preprogrammed motor response, making them less efficient inhibitors.

Examination of descriptive statistics revealed a significant age difference between the SS group and the two other groups by almost 10 years. Older adults have been found to perform poorer than younger on tasks involving inhibition (Kramer, Humphrey, Larish, Logan, & Strayer 1994), thus it is possible that age may have contributed to the differences in stop signal reaction time. The results showed a significant effect of age on SSRT, and the effect of genotype was insignificant. According to these results the difference between our groups is more strongly related to age than to genotype.

Different distribution of genotypes in the population according to age seems unlikely. There was no obvious aspect of the sampling criteria that could explain the age difference between groups in the sample, thus we assume age difference between groups is a product of mere coincidence. Compared to the homozygous SS group and the LL group the heterozygous SL group achieved an intermediate stop signal reaction time closely resembling the SS group, suggesting a relatively smaller impact of the L_A allele in heterozygous carriers. This is in line with results reported by Lesch et al. (1996) suggesting a functional dominance of the S allele over the L allele, and justifies collapsing SS and SL groups in analysis. In the literature of 5-HTTLPR and impulsivity there is no consensus about how to classify the different genotypes. Several researchers have applied the dominance model without

consideration of the SNP. When data were reanalyzed adhering to this model and controlled for age, genotype approached significance.

A problem with classifying genotypes without considering the SNP is that the LL group comprises both L_A and L_G alleles (L_AL_A, L_AL_G and L_GL_G genotypes). The stop signal reaction time of the LL group is here confounded by low-expressive L_G alleles functionally similar to S alleles (Hu et al., 2006), possibly reducing the observed difference between homozygous SS and LL groups and concealing the potential effect of genotype on inhibition. The triallelic model omits this problem by limiting the LL group to L_AL_A genotypes. The triallelic model is therefore preferable in studies of the effect of 5-HTTLPR on behavior. When analyzed with the triallelic dominance model, both genotype and age were significantly associated with inhibition. A possible limitation of this model is that L_GL_A genotypes are classified as low-expressive, even though the dominance between L_G and L_A alleles remains uncertain.

In a comparable study Clark et al. (2005) found no association between 5-HTTLPR and inhibition. An important difference between Clark et al.'s study and the present study is the classification of genotypes. Clark et al. used the biallelic model, not considering the SNP in the L allele. The functionality of the L_G allele may have confounded the results and could explain why Clark et al. failed to detect an association. Another important limitation was the relatively small number of participants (n=42) tested by Clark et al. Assuming a small effect size of genetic variance on behavior, a larger sample may be required to detect a significant effect. The doubled sample size and the triallelic classification of genotypes may have contributed to the detection of an existing effect of 5-HTTLPR genotype on inhibition in our study.

A subordinate purpose of our study was to investigate a possible genotype by sex interaction effect on impulsivity. The results from the stop signal task indicated no such interaction.

5-HTTLPR and Response Style

The results from CPT showed no significant association between the polymorphism and false alarms or β. Our results suggest that 5-HTTLPR is not related to impulsivity measured by the CPT. However, CPT is similar to the visual comparison task applied by Paaver et al. (2007) who found a significant association between impulsivity and 5-HTTLPR. They applied a test where participants were instructed to respond to whether two complex visual figures were identical or not, and calculated a measure of impulsivity based on speed accuracy tradeoff.

Subjects with shorter response time and higher error rates were regarded as more impulsive. Even though this test is similar to the CPT in respect of detecting identical stimuli, the task in Paaver et al. lacks the element of rapid response. This element provokes uncertainty in the CPT test due to incomplete processing, and how participants handle this uncertainty seems to be a conceptually different phenomenon from the speed accuracy tradeoff. Perhaps the CPT and the visual comparison task tap different aspects of impulsivity. This may explain the diverging results between this study and Paaver et al.'s.

No significant genotype x sex interaction was found on the CPT variables. However, graphic display of β means indicated an opposite pattern for men and women in the numbers condition. Whereas S allele was associated with impulsive response style in men (β lower than 1), S allele in women was associated with cautious response style (β higher than 1). Walderhaug et al. (2007) found the same sex-dependent effects on response style by experimentally induced 5-HT deprivation. They suggested that different function in the serotonin system for men and women may explain the discrepancy in distribution of psychiatric disorders between the sexes. Men have a higher prevalence of disorders related to impulse control and women a higher prevalence of mood and anxiety disorders (Holden, 2005; Kessler et al., 2005). The pattern observed in our data on the numbers condition suggests that the same effect could be related to 5-HTTLPR function in the serotonin system.

Means varied around 1 in the β numbers condition. In contrast, values exceeded 1 for all groups in the β shapes condition, reflecting cautious response styles independent of sex or genotype. A possible explanation for the high β shapes values is that the task was too difficult and led subjects to careful responding. In comparison, it appears that the degree of difficulty in the numbers condition was more apt to capture differences in response style.

General Discussion

All subjects were thoroughly evaluated for mental disorders, and all participants fulfilling any current or previous mental disorder by SCID I and II criteria were excluded. Additionally, Beck Depression Inventory scores were available for all subjects. A common cut-off criteria for healthy subjects in research is exclusion of BDI score larger than 10. The reason for excluding subjects who show symptoms of depression is that depression is significantly and robustly associated with impairment in a variety of cognitive abilities (Landrø & Andersson, 2008), which makes depression a confounding element when studying cognitive performance. Diagnostic criteria on the SCID I module for affective disorders were relied on as exclusion criteria in relation to depression. Five of the 87 healthy subjects reached BDI

scores larger than 10 (scores 11, 11, 13, 16 and 16) which indicates that they had experienced some symptoms of depression during the last two weeks before testing. These subjects were included as they did not fulfill diagnostic criteria on the SCID I module for affective disorders. Informal investigation of data indicated that exclusion of these five subjects would have made very little impact on mean stop signal reaction times, thus depressive symptoms were not included as a covariate in our analysis.

An important question is whether our sampling criteria, excluding all subjects with present or previous psychiatric disorders and drug abuse, could reduce the probability of detecting a true association, thus decreasing the validity. Cannon and Keller (2006) argue that it is not necessary to screen for disorders to study the genetics of endophenotypes because endophenotypes should vary continuously in the general population. The endophenotype should vary with level of genetic risk independent of the individual's symptoms or diagnosis.

Due to the several intermediate levels of influence between the genetic level and the cognitive processing level, it should be expected that any single genetic variation can only explain a modest proportion of variance in an endophenotype on the level of cognitive processing. Accordingly, multiple other determinants will be needed to explain the large part of variance in our targeted endophenotype. Perhaps the exclusion according to strict clinical criteria leaves us with subjects who possess such protective qualities in other genotypes that the effect of 5-HTTLPR on inhibitory control and response style appears less potent than it is in the general population.

An advantage of neuropsychological measures is that they are not vulnerable to subjective judgment or evaluation of behavior, such as may often be the case when subjects answer questionnaires. The tests used were also computerized to reduce experimenter bias. The theoretical backgrounds for SST and CPT suggest that although they have both been used to assess impulsivity, they measure different aspects. In the stop signal task impulsivity is operationalized as stop signal reaction time. In line with Logan's (1994; Logan et al., 1997) race model, inhibition of response occurs when the inhibition process is faster than the go process. These processes are seen as parallel processes. Other researchers have argued that response inhibition involves more than one process and that the stop signal task measures a global inhibition process. The global inhibition process may be involved in all inhibition and may be moderated by an additional disinhibitory or selective control process when selective inhibition is required (Aron & Verbruggen, 2008; Coxon et al., 2007; De Jong et al., 1990). In an executive function model inhibition is one of three postulated functions, the others being mental set shifting, and information updating and monitoring. These functions may be

considered separately, though they also seem to work as a unity. Inhibition has been proposed to be both a separate function, and a global inhibitory function, moderating other executive functions (Miyake et al., 2000). Results implying both a global and a selective inhibition process seem to support this model. Yet inhibition may not only stop preprogrammed responses but also responses that are not preprogrammed. One example is in a choice situation where two or more responses are possible and the individual has to choose one of the response alternatives. Inhibition seems to involve various processes and have several important functions in cognition. The stop signal task is a measure that captures an essential element of the phenomenon.

Whereas the stop signal task has been developed from a theory of cognitive processes, the continuous performance test has been evolved from signal detection theory, traditionally more concerned with perception. In the CPT the rapid response is an important element, requiring a decision of whether two stimuli are equal or not, when stimuli processing is incomplete. How subjects react to this uncertainty is assumed to reflect impulsive or cautious response style. Ability and willingness to decide and act rapidly or preference for careful consideration, planning and security are manifested as tendencies to over- or under respond. Response style is complex because it involves several processes. Planning, rapid decision making and memory are processes involved in response style. These are also processes moderated by executive functions. Thus variety in executive functions may influence response style. The response style measure is a broader concept than the response inhibition measure and it may be easier to detect an association between a genotype and a more narrowly defined concept.

The results suggest that the S and L_G alleles of the 5-HTTLPR are associated with slower and less efficient response inhibition compared to the L_A allele. However, a common issue in correlation studies is the lack of control over extraneous variables that may have influenced the observed association. The effect of 5-HTTLPR on impulsivity has been found to interact with blood platelet monoamine oxidase (MAO) activity (Paaver et al., 2007) and with the MAOA gene polymorphism (Passamonti et al., 2008). A variable number tandem repeat region (VNTR) of the 5-HTT gene, and several genes related to the dopamine system have also been associated with impulsivity (Aluja, Garcia, Blanch, De Lorenzo, & Fibla, 2009; Aron & Poldrack, 2005; Manuck et al., 2000). This study was limited to investigating the effect of 5-HTTLPR and did not explore these interactions. Thus it is possible that 5-HTTLPR is also associated with inhibition through interaction with other genes.

During testing some participants reported experiencing both the stop signal task and the continuous performance test as very difficult. Although testers were instructed to encourage subjects and validate experience of frustration and annoyance, test experience may have led some subjects to sabotage the test by responding randomly. Others may have given up trying to respond correctly thus responded randomly or not at all. The stop signal task was designed to adjust the stop signal delay during the first half of the test to a point where the subject was able to inhibit correctly in 50% of the trials. This should have reduced frustration to some extent because the subject experienced a proportion of successful responses. Frustration and annoyance may have been greater in the continuous performance test because level of difficulty was not adjusted to subjects' performance in this task. On the other hand, Bowman, Evans and Turnbull (2005) found that frustration did not affect cognitive behavioral performance. Outliers in our data were presumed to reflect random or erroneous task completion and were therefore excluded from statistical analysis to prevent confounding of the results. Although outliers were excluded from our statistical analyses to reduce error variance, we cannot be sure that experience of the test did not influence subjects' performance. To enable control over possible effects of frustration systematic observations of how the tests were experienced could have been registered.

A limitation in the analysis of our data is the lack of statistical power due to small group sizes when data were classified according to both genotype and sex. The validity of observations in small groups is generally more compromised by random errors. The β data were characterized by small groups and large within-group variances, possibly confounding a between-group difference. Even though an effect of genotype on response style was not found, the lack of statistical power in combination with few comparable studies necessitate further investigation to reject the hypothesis of an effect of the serotonin transporter polymorphism on response style. The sample size does not seem to have limited the SST results to the same extent, however a larger sample would increase statistical power and could have revealed nuances in stop signal reaction time between SL and SS groups.

The stop signal task has been found to differentiate pathological impulsive from healthy populations. Poorer inhibitory control, reflected by prolonged stop signal reaction time, has been found in children with ADHD (Lijffijt, Kenemans, Verbaten, & Engeland, 2005), various forms of substance abuse (Goudriaan et al., 2006; Fillmore & Rush, 2002; Monterosso, Aron, Cordova, Xu & London, 2005) and in people with obsessive compulsive disorder (Chamberlain, Fineberg, Blackwell, Robbins, & Sahakian, 2006) and Tourette's syndrome (Goudriaan et al., 2006). In a longitudinal study, impaired response inhibition in

adolescence was found to predict problem drinking and drug use independent of IQ and ADHD (Nigg et al. 2006), indicating that impaired response inhibition constitutes a causal risk factor and is not just a result of alcohol and drug abuse. Impaired inhibitory control has also been found in siblings of children with ADHD, implying that this characteristic aggregates in families and may be associated with a genetic vulnerability (Schachar et al. 2005). Based on these findings response inhibition has been proposed an endophenotype for various disorders related to impaired impulse control. The results of the present study suggest that S and L_G alleles constitute vulnerabilities for impaired inhibition, forming a diathesis in disorders related to impaired impulse control.

Healthy female adults, homozygote for the short allele, have been found to be biased towards negative information. They were more sensitive to sad faces than to other facial expressions when tested within the context of a working memory task (Landrø et al., 2009). This seems to support a cognitive vulnerability model for development of depression, where inability to fully inhibit previously relevant negative information lead to prolonged activation of negative information in working memory, resulting in sustained negative affect and recurring negative thoughts.

Attentional bias for negative information has been found in individuals with clinical depression and has been explained by difficulty in disengaging from negative information once it has become the focus of the individual's attention (Gotlib et al., 2004). Thus depressed individuals seem to have impaired inhibition for negative information. Poorer inhibitory control in individuals with short allele in combination with bias towards negative information found in female SS carriers may render individuals with a short allele more vulnerable for depression. In line with this hypothesis short allele has been associated with increased risk for development of depression in people with experience of stressful life events (Caspi et al., 2003). It is noteworthy that even after excluding depressed individuals by diagnostic criteria, we found relatively higher depression scores, based on BDI, in the groups with the S allele.

The endophenotype model (Cannon & Keller, 2006) is a tool for clearer understanding of intermediate processes between genotypes and syndromes. This model is useful when investigating the association between the different alleles of the serotonin transporter polymorphism and impulsivity. The results of the present study indicate that short allele is associated with poorer inhibition. According to the endophenotype model the short allele may contribute to depression and impulse control disorders by mediating central inhibitory control.

Future research should investigate the association between the serotonin transporter polymorphism and inhibition of negative emotional stimuli. Including individuals with clinical depression may increase understanding of how impaired function in inhibitory control influences cognitive processing in normal and depressed people. In the present study, no significant interaction between genotype and sex was found. Bias towards negative stimuli found in SS women, and higher prevalence of women suffering from mood and anxiety disorders, still suggest that possible sex differences in serotonin functioning should be further explored.

Concluding Remarks

The present study links the serotonin transporter polymorphism to inhibitory control. Short allele was associated with poorer performance on a theoretically and empirically validated measure of inhibition.

Inhibition is a central component of executive functions and impairment in these functions are associated with various psychological disorders. Through its effect on inhibition, the short allele may constitute a genetic vulnerability for impulse control disorders and depression.

REFERENCES

Aluja, A., Garcia, L. F., Blanch, A., De Lorenzo, D., & Fibla, J. (2009). Impulsive-disinhibited personality and serotonin transporter gene polymorphisms: Association study in an inmate's sample. *Journal of Psychiatric Research, 43,* 906-914.

American Psychiatric Association (2000). *Diagnostic and statistical manual of mental disorders, (4th ed., Text Revision).* Washington, DC: American Psychiatric Association.

Aron A. R., Dowson, J. H., Sahakian, B. J., & Robbins, T. W. (2003). Methylphenidate improves response inhibition in adults with attention-deficit/hyperactivity disorder. *Biological Psychiatry, 54,* 1465-1468.

Aron, A. R., & Poldrack, R. A. (2005). The cognitive neuroscience of response inhibition: Relevance for genetic research in attention-deficit/hyperactivity disorder. *Biological Psychiatry, 57,* 1285-1292.

Aron, A. R., & Verbruggen, F. (2008). Stop the presses: Dissociating a selective from a global mechanism for stopping. *Psychological Science, 19,* 1146-1153.

Baca-García, E., Vaquero, C., Diaz-Sastre, C., Saiz-Ruiz, J.,Fernández-Piqueras, J., & de Leon, J. (2002) A gender-specific association between the serotonin transporter gene and suicide attempts. *Neuropsychopharmachology, 26,* 692-695.

Baddeley, A. (1996). Exploring the central executive. *Quarterly Journal of Experimental Psychology, 49A,* 5-28.

Barratt, E. S. (1985). Impulsiveness subtraits: Arousal and information processing. In J. T. Spence & C. E. hard (Eds.), *Motivation, emotion, and personality* (pp. 137-146). Amsterdam, Holland: Elsevier Science.

Barkley, R. A. (1999). Response inhibition in attention-deficit hyperactivity disorder. *Mental Retardation and Developmental Disabilities Research Reviews, 5,* 177-184.

Bell, C. J., Hood, S. D., & Nutt, D. J. (2005). Acute tryptophan depletion. Part II: Clinical effects and implications. *Australian and New Zealand Journal of Psychiatry, 39,* 565-574.

Bowman, C. H., Evans, C. E. Y., & Turnbull, O. H. (2005). Artificial time constraints on the Iowa Gambling Task: The effects on behavioural performance and subjective experience. *Brain and Cognition, 57,* 21-25.

Cambridge Cognition Limited (2006). CANTABeclipse™ software battery of neuropsychological tests. Cambridge, England: Cambridge Cognition Limited.

Cannon, T. D., & Keller, M. C. (2006). Endophenotypes in the genetic analyses of mental disorders. *Annual Review of Clinical Psychology, 2,* 267-290.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., et al. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science, 301,* 386-389.

Chamberlain, S. R., Fineberg, N. A., Blackwell, A. D., Robbins, T. W., & Sahakian, B. J. (2006). Motor inhibition and cognitive flexibility in obsessive-compulsive disorder and trichotillomania. *American Journal of Psychiatry, 163,* 1282-1284.

Chamberlain, S. R., Fineberg, N. A., Menzies, L. A., Blackwell, A. D., Bullmore, E. T., Robbins, T. W., et al. (2007). Impaired cognitive flexibility and motor inhibition in unaffected first-degree relatives of patients with obsessive-compulsive disorder. *American Journal of Psychiatry, 164,* 335-338.

Clark, L., Roiser, J. P., Cools, R., Rubinsztein, D. C., Sahakian, B. H., & Robbins, T. W. (2005). Stop signal response inhibition is not modulated by tryptophan depletion or the serotonin transporter polymorphism in healthy volunteers: Implications for the 5-HT theory of impulsivity. *Psychopharmacology, 182,* 570-578.

Conners, C. K., Epstein, J. N., Angold, A., & Klaric, J. (2002). Continuous Performance Test performance in a normative epidemiological sample. *Journal of Abnormal Child Psychology, 31,* 555-562.

Cornblatt, B. A., Risch, N. J., Faris, G., Friedman, D., & Erlenmeyer-Kimling, L. (1988). The Continuous Performance Test, identical pairs version (CPT-IP): I. New findings about sustained attention in normal families. *Psychiatry Research, 26,* 223-238.

Coxon, J. P., Stinear, C. M., & Byblow, W. D. (2007). Selective inhibition of movement. *Journal of Neurophysiology, 97,* 2480-2489.

De Jong, R., Coles, M. G. H., Logan, G. D., & Gratton, G. (1990). In search of the point of no return: The control of response processes. *Journal of Experimental Psychology: Human Perception and Performance, 16,* 164-182.

Dickman, S. J. (1990). Functional and dysfunctional impulsivity: Personality and cognitive correlates. *Journal of Personality and Social Psychology, 58,* 95-102.

Dickman, S. J. (1993). Impulsivity and information processing. In W. G. McCown, J. L. Johnson, & M. B. Shure (Eds.), *The impulsive client: Theory, research and treatment* (pp. 151-184). Washington, D.C.: American Psychological Association.

Dougherty, D. M., Bjork, J. M., Harper, R. A., Marsh, D. M., Moeller, F. G., Mathias, C. W., et al. (2003). Behavioral impulsivity paradigms: A comparison in hospitalized adolescents with disruptive behavior disorders. *Journal of Child Psychology and Psychiatry, 44,* 1145-1157.

Dougherty, D. M., Moeller, F. G., Steinberg, J. L., Marsh, D. M., Hines, S. E., & Bjork, J. M. (1999). Alcohol increases commission error rates for a continuous performance test. *Alcoholism: Clinical and Experimental Research, 23,* 1342-1351.

Du, L., Faludi, G., Palkovits, M., Demeter, E., Bakish, D., Lapierre, Y. D., et al. (2000). Frequency of long allele in serotonin transporter gene is increased in depressed suicide victims. *Biological Psychiatry, 46,* 196-201.

Epstein, J. N., Erkanli, A., Conners, C. K., Klaric, J., Costello, J. E., & Angold, A. (2003). Relations between Continuous Performance Test performance measures and ADHD behaviors. *Journal of Abnormal Child Psychology, 31,* 543-554.

Evenden, J. L. (1999). Varieties of impulsivity. *Psychopharmacology, 146,* 348-361.

Eysenck, S. B. G. (1993). The I_7: Development of a measure of impulsivity and its relationship to the superfactors of personality. In: W. G. McCown, J. L. Johnson, & M. B. Shure (Eds.), *The impulsive client: theory, research and treatment* (pp. 141-149). Washington, D.C: American Psychological Association.

Fairbanks, L. A., Melega, W. P., Jorgensen, M. J., Kaplan, J. R., & McGuire, M. T. (2001). Social impulsivity inversely associated with CSF 5-HIAA and fluoxetine exposure in vervet monkeys. *Neuropsychopharmacology, 24,* 370-378.

Faraj, B. A., Olkowski, Z. L., & Jackson, R. T. (1994). Expression of high-affinity serotonin transporter in human lymphocytes. *International Journal of Immunopharmacology, 16,* 561-567.

Fillmore, M. T., & Rush, C. R. (2002). Impaired inhibitory control of behavior in chronic cocaine users. *Drug and Alcohol Dependence, 66,* 265-273.

Friedman, N. P., Miyake, A., Young, S. E., DeFries, J. C., Corley, R. P., & Hewitt, J. K. (2008). Individual differences in executive functions are almost entirely genetic in origin. *Journal of Experimental Psychology: General, 137,* 201-225.

Gelernter, J., Kranzler, H., & Cubells, J. F. (1997). Serotonin transporter protein (SLC6A4) allele and haplotype frequencies and linkage disequilibria in African- and European-American and Japanese populations and in alcohol-dependent subjects. *Human Genetics, 101,* 243-246

Goldberg, L. R. (1990). An alternative "description of personality": The Big-Five factor structure. *Journal of Personality and Social Psychology, 59,* 1216-1229.

Gotlib, I. H., Krasnoperova, E., Yue, D. N. & Joormann, J. (2004). Attentional biases for negative interpersonal stimuli in clinical depression. *Journal of Abnormal Psychology, 113,* 127-135

Gottesman, I. I., & Gould, T. G. (2003). The endophenotype concept in psychiatry: Etymology and strategic intentions. *American Journal of Psychiatry, 160,* 636-645.

Goudriaan, A. E., Oosterlaan, J., de Beurs, E., & van den Brink, W. (2006). Neurocognitive functions in pathological gambling: A comparison with alcohol dependence, Tourette syndrome and normal controls. *Addiction, 101,* 534-547.

Greenberg, B. D., Li, Q., Lucas, F., Hu, S., Sirota, L. A., Benjamin, J., et al. (2000). Association between the serotonin transporter promoter polymorphism and personality traits in a primarily female population sample. *American Journal of Medical Genetics (Neuropsychiatric Genetics), 96,* 202-216.

Greene, C. M., Braet, W., Johnson, K. A., & Bellgrove, M. A. (2008). Imaging the genetics of executive function. *Biological Psychology, 79,* 30-42.

Grunebaum, H., Weiss, J. L., Gallant, D., & Cohler, B. J. (1974). Attention in young children of psychotic mothers. *American Journal of Psychiatry, 131,* 887-891.

Halperin, J. M., Wolf, L., Greenblatt, E. R., & Young, G. (1991). Subtype analysis of commission errors on the Continuous Performance Test in children. *Developmental Neuropsychology, 7,* 207-217.

Heils, A., Teufel, A., Petri, S., Stöber, G., Riederer, P., Bengel, D., et al. (1996) *Journal of Neurochemistry, 66,* 2621-2624.

Heinz, A., Jones, D. W., Mazzanti, C., Goldman, D., Ragan, P., Hommer, D., et al. (2000). A relationship between serotonin transporter genotype and in vivo protein expression and alcohol neurotoxicity. *Biological Psychiatry, 47,* 643-649.

Holden, C. (2005). Sex and the suffering brain. *Science, 308,* 1574-1577.

Hranilovic, D., Stefulj, J., Schwab, S., Borrmann-Hassenbach, M., Albus, M., Jernej, B., et al. (2004). Serotonin transporter promoter and intron 2 polymorphisms: Relationship between allelic variants and gene expression. *Biological Psychiatry, 55,* 1090-1094.

Hu, X., Zhu, G., Lipsky, R. H., & Goldman, D. (2004). HTTLPR allele expression is codominant, correlating with gene effects on fMRI and SPECT imaging intermediate phenotypes, and behavior [Abstract]. *Biological Psychiatry, 55 (Suppl 1),* 191S.

Hu, X.-Z., Lipsky, R. H., Zhu, G., Akhtar, L. A., Taubman, J., Greenberg, B. D., et al. (2006). Serotonin transporter protein gain-of-function genotypes are linked to obsessive-compulsive disorder. *American Journal of Human Genetics, 78,* 815-826.

Jacob, C. P., Strobel, A., Hohenberger, K., Ringel, T., Gutknecht, L., Reif, A., et al. (2004). Association between allelic variation of serotonin transporter function and neuroticism in anxious cluster C personality disorders. *American Journal of Psychiatry, 161,* 569-572.

Jacobsen, L. K., Staley, J. K., Zoghbi, S., Seibyl, J. P., Kosten, T. R., Innis, R. B., et al. (2000). Prediction of dopamine transporter binding availability by genotype: A preliminary report. *American Journal of Psychiatry, 157,* 1700-1703.

Jurado, M. B., & Rosselli, M. (2007). The elusive nature of executive functions: A review of our current understanding. *Neuropsychology Review, 17,* 213-233.

Katsuragi, S., Kunugi, H., Sano, A., Tsutsumi, T., Isogawa, K., Nanko, S., et al. (1999). Association between serotonin transporter gene polymorphism and anxiety-related traits. *Biological Psychiatry, 45,* 368-370.

Keilp, J. G., Herrera, J., Strizke, P., & Cornblatt, B. A. (1997). The Continuous Performance Test, identical pairs version (CPT-IP): III. Brain functioning during performance of numbers and shapes subtasks.

Kent, L., Doerry, U., Hardy, E., Parmar, R., Gingell, K., Hawi, Z., et al. (2002). Evidence that variation at the serotonin transporter gene influences susceptibility to attention deficit hyperactivity disorder (ADHD): Analysis and pooled analysis. *Molecular Psychiatry, 7,* 908-912.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. *Archives of General Psychiatry, 62,* 593-602.

Kramer, A. F., Humphrey, D. G., Larish, J. F., Logan, G. D., & Strayer, D. L. (1994). Aging and inhibition: Beyond a unitary view of inhibitory processing in attention. *Psychology and Aging, 9,* 491-512.

Landrø, N. I., & Andersson, S. (2008). Nevropsykologiske aspekter ved stemningslidelser. *Tidsskrift for Norsk Psykologforening, 45,* 1155-1163.

Landrø, N. I., Jonassen, R., Lyche, P., Neumeister, A., Stiles, T. C., & Endestad, T. (2009). Mael-female seroronin transporter polymophisms and emotion processing. *Frontiers in Neuroscience, 3,* 244-245.

Lane, S. D., Cherek, D. R., Rhoades, H. M., Pietras, C. J., & Tcheremissine, O. V. (2003). Relationships among laboratory and psychometric measures of impulsivity: Implications in substance abuse and dependence. *Addictive Disorders and Their Treatment, 2,* 33-40.

Lauder, J. M. (1993). Neurotransmitters as growth regulatory signals: role of receptors and second messengers. *Trends in Neurosciences, 16,* 233-239.

Lee, J.-H., Kim, H.-T., & Hyun, D.-S. (2003). Possible association between serotonin transporter promoter region polymorphism and impulsivity in Koreans. *Psychiatry Research, 118,* 19-24.

Lesch, K.-P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science, 274,* 1527-1531.

Lesch, K.-P., & Gutknecht, L. (2005). Pharmacogenetics of the serotonin transporter. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 29,* 1062-1073.

Lesch, K.-P., & Mössner, R. (1998). Genetically driven variation in serotonin uptake: Is there a link to affective spectrum, neurodevelopmental, and neurodegenerative disorders? *Biological Psychiatry, 44,* 179-192.

Lijffijt, M., Kenemans, J. L., Verbaten, M. N., & van Engeland, H. (2005). A meta-analytic review of stopping performance in attention-deficit/hyperactivity disorder: Deficient inhibitory motor control? *Journal of Abnormal Psychology, 114,* 216-222.

Lim, J.-E., Papp, A., Pinsonneault, J., Sadée, W., & Saffen, D. (2006). Allelic expression of serotonin transporter (SERT) and mRNA in human pons: Lack of correlation with the polymorphism SERTLPR. *Molecular Psychiatry, 11,* 649-662.

Little, K. Y., McLaughlin, D. P., Zhang, L., Livermore, C. S., Dalack, G. W., McFinton, P. R., et al. (1998). Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. *American Journal of Psychiatry, 155,* 207-213.

Logan, G. D. (1994). On the ability to inhibit thought and action: A users' guide to the stop signal paradigm. In D. Dagenbach, & T. H. Carr (Eds.), *Inhibitory processes in attention, memory and language* (pp. 189-239). San Diego, CA: Academic Press.

Logan, G. D., & Cowan, W. B. (1984). On the ability to inhibit thought and action: A theory of an act of control. *Psychological Review, 91,* 295-327.

Logan, G. D., Cowan, W. B., & Davis, K. A. (1984). On the ability to inhibit simple and choice reaction time responses: A model and a method. *Journal of Experimental Psychology: Human Perception and Performance, 10,* 276-291.

Logan, G. D., Schachar, R. J., & Tannock, R. (1997). Impulsivity and inhibitory control. *Psychological Science, 8,* 60-64.

Lyons-Ruth, K., Holmes, B. M., Sasvari-Szekely, M., Ronai, Z., Nemoda, Z., & Pauls, D. (2007). Serotonin transporter polymorphism and borderline or antisocial traits among low-income young adults. *Psychiatric Genetics, 17,* 339-343.

Mackworth, J. F., & Taylor M. M. (1963). The d' measure of signal detectability during vigilance-like situations. *Canadian Journal of Psychology, 17,* 302-325.

Manor, I., Eisenberg, J., Tyano, S., Sever, Y., Cohen, H., Ebstein, R. P., et al. (2001). Family-based association study of the serotonin transporter promoter region polymorphism (5-HTTLPR) in attention deficit hyperactivity disorder. *American Journal of Medical Genetics (Neuropsychiatric Genetics), 105,* 91-95.

Manuck, S. B., Flory, J. D., Ferrell, R. E., Mann, J. J., & Muldoon, M. F. (2000). A regulatory polymorphism of the monoamine oxidase-A gene may be associated with variability in aggression, impulsivity, and central nervous system serotonergic responsivity in nonpatient sample. *Neuropsychopharmacology, 19,* 9-23.

McCrae, R. R., & Costa, P. T. Jr. (1997). Personality trait structure as a human universal. *American Psychologist, 52,* 509-516.

Menzies, L., Achard, S., Chamberlain, S. R., Fineberg, N., Chen, C.-H., del Campo, N., et al. (2007). Neurocognitive endophenotypes of obsessive-compulsive disorder. *Brain, 130,* 3223-3226.

Miyake, A., Friedman, N. P., Emerson, M. J., Witzki, A. H., Howerter, A., & Wager, T. D. (2000). The unity and diversity of executive functions and their contributions to complex "frontal lobe" tasks: A latent variable analysis. *Cognitive Psychology, 41,* 49-100.

Monteresso, J. R., Aron, A. R., Cordova, X., Xu, J., & London, E. D. (2005). Deficits in response inhibition associated with chronic methamphetamine abuse. *Drug and Alcohol Dependence, 79,* 273-277.

Nakamura, M., Ueno, S., Sano, A., & Tanabe, H. (2000). The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. *Molecular Psychiatry, 5,* 32-38.

Neumeister, A., Hu, X.-Z., Luckenbaugh, D. A., Schwarz, M., Nugent, A. C., Bonne, O., et al. (2006). Differential effects of 5-HTTLPR genotypes on the behavioral and neural responses to tryptophan depletion in patients with major depression and controls. *Archives of General Psychiatry, 63,* 978-986.

Newman, J. P., Widom, C. S., & Nathan, S. (1985). Passive avoidance in syndromes of disinhibition: Psychopathy and extraversion. *Journal of Personality and Social Psychology, 48,* 1316-1327.

Ni, X., Chan, K., Bulgin, N., Sicard, T., Bismil, R., McMain, S., et al. (2006). Association between serotonin transporter gene and borderline personality disorder. *Journal of Psychiatric Research, 40,* 448-453.

Nigg, J. T., Wong, M. M., Martel, M. M., Jester, J. M., Puttler,L. I., Glass, J. M., et al. (2006). Poor response inhibition as a predictor of problem drinking and illicit drug use in adolescents at risk for alcoholism and other substance abuse disorders. *Journal of the American Academy of Child and Adolescent Psychiatry, 45,* 468-475.

Nobile, M., Begni, B., Giorda, R., Frigerio, A., Marino, C., Molteni, M., et al. (1999). Effects of serotonin transporter functionality in depressed children and adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry, 38,* 1396-1402.

Osman, A., Kornblum, S., & Meyer, D. E. (1986). The point of no return in choice reaction time: Controlled and ballistic stages of response preparation. *Journal of Experimental Psychology: Human Perception and Performance, 12,* 243-258.

Paaver, M., Kurrikoff, T., Nordquist, N., Oreland, L., & Harro, J. (2008). The effect of 5-HTT gene promoter polymorphism on impulsivity depends on family relations in girls. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 32,* 1263-1268.

Paaver, M., Nordquist, N., Parik, J., Harro, M., Oreland, L., & Harro, J. (2007). Platelet MAO activity and the 5-HTT gene promoter polymorphism are associated with impulsivity and cognitive style in visual information processing. *Psychopharmacology, 194,* 545-554.

Parsey, R. V., Hastings, R. S., Oquendo, M. A., Hu, X., Goldman, D., Huang, Y., et al. (2006). Effect of a triallelic functional polymorphism of the serotonin-transporter-linked promoter region on expression of serotonin transporter in the human brain. *American Journal of Psychiatry, 163,* 48-51.

Pascual, J. C., Soler, J., Barrachina, J., Campins, M. J., Alvarez, E., & Pérez, V. (2008). Failure to detect an association between the serotonin transporter gene and borderline personality disorder. *Journal of Psychiatric Research, 42,* 87-88.

Passamonti, L., Cerasa, A., Gioia, M. C., Magariello, A., Muglia, M., Quattrone, A., et al. (2008). Genetically dependent modulation of serotonergic inactivation in the human prefrontal cortex. *Neuroimage, 40,* 1264-1273.

Patton, J. H., Stanford, M. S., & Barratt, E. S. (1995). Factor structure of the Barratt Impulsiveness Scale. *Journal of Clinical Psychology, 51,* 768-774.

Praschak-Rieder, N., Kennedy, J., Wilson, A. A., Hussey, D., Boovariwala, A., Willeit, M., et al. (2007). Novel 5-HTTLPR allele associates with higher serotonin transporter binding in putamen: A [^{11}C] DASB positron emission tomography study. *Biological Psychiatry, 62,* 327-331.

Preuss, U. W., Soyka, M., Bahlmann, M., Wenzel, K., Behrens, S., de Jonge, et al. (2000). Serotonin transporter gene regulatory region (5-HTTLPR), [^{3}H]paroxetine binding in healthy control subjects and alcohol-dependent patients and their relationships to impulsivity. *Psychiatry Research, 96,* 51-61.

Retz, W., Thome, J., Blocher, D., Baader, M., & Rössler, M. (2002). Association of attention deficit hyperactivity disorder-related psychopathology and personality traits with the serotonin transporter promoter region polymorphism. *Neuroscience Letters, 319,* 133-136.

Retz, W., Freitag, C. M., Retz-Junginger, P., Wenzler, D., Schneider, M., Kissling, C., et al. (2008). A functional serotonin transporter gene polymorphism increases ADHD symptoms in delinquents: Interaction with adverse childhood environment. *Psychiatry Research, 158,* 123-131.

Reynolds, B., Ortengren, A., Richards, J. B., & de Wit, H. (2006). Dimensions of impulsive behavior: Personality and behavioral measures. *Personality and Individual Differences, 40,* 305-315.

Sakado, K., Sakado, M., Muratake, T., Mundt, C., & Someya, T. (2003). A psychometrically derived impulsive trait related to a polymorphism in the serotonin transporter gene-linked polymorphic region (5-HTTLPR) in a Japanese nonclinical population: Assessment by the Barratt Impulsiveness Scale (BIS). *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics), 121B,* 71-75.

Schachar, R. J., Crosbie, J., Barr, C. L., Ornstein, T. J., Kennedy, J., Malone, M., et al. (2005). Inhibition of motor responses in siblings concordant and discordant for attention deficit hyperactivity disorder. *American Journal of Psychiatry, 162,* 1076-1082.

Seeger, G., Schloss, P., & Schmidt, M. H. (2001). Functional polymorphism within the promotor of the serotonin transporter gene is associated with severe hyperkinetic disorders. *Molecular Psychiatry, 6,* 235-238.

Shioe, K., Ichimiya, T., Suhara, T., Takano, A., Sudo, Y., Yasuno, F., et al. (2003). No association between genotype of the promoter region of serotonin transporter gene and serotonin transporter binding in human brain measured by PET. *Synapse, 48,* 184-188.

Sirota, L. A., Greenberg, B. D., Murphy, D. L., & Hamer, D. H. (1999). Non-linear association between the serotonin transporter promoter polymorphism and neuroticism: A caution against using extreme samples to identify quantitative trait loci. *Psychiatric Genetics, 9,* 35-38.

Sjöberg, R. L., Nilsson, K. W., Nordquist, N., Öhrvik, J., Leppert, J., Lindström, L., et al. (2006). Development of depression: sex and the interaction between environment and a promoter polymorphism of the serotonin transporter gene. *International Journal of Neuropsychopharmacology, 9,* 443-449.

Slaats-Willemse, D., Swaab-Barneveld, H., de Sonneville, L., van der Muelen, E., & Buitelaar, J. (2003). Deficient response inhibition as a cognitive endophenotype of ADHD. *Journal of the American Academy of Child & Adolescent Psychiatry, 42,* 1242-1248.

Stein, M. B., Seedat, S. & Gelernter, J. (2006). Serotonin transporter gene promoter polymorphism predicts SSRI response in generalized social anxiety disorder. *Psychopharmacology, 187,* 68 –72.

Stoltenberg, S. F., Twitchell, G. R., Hanna, G. L., Cook, E. H., Fitzgerald, H. E., Zucker, R. A., et al. (2002). Serotonin transporter promoter polymorphism, peripheral indexes of serotonin function, and personality measures in families with alcoholism. *American Journal of Medical Genetics (Neuropsychiatric Genetics), 114,* 230-234.

Swann, A. C., Bjork, J. M., Moeller, F. G., & Dougherty, D. M. (2002). Two models of impulsivity: Relationship to personality traits and psychopathology. *Biological Psychiatry, 51,* 988-994.

Swets, J. A., Tanner, W. P., & Birdsall, T. G. (1961). Decision processes in perception. *Psychological Review, 68,* 301-340.

Tadić, A., Victor, A., Başkaya, Ö., von Cube, R., Hoch, J., Kouti, I., et al. (2009). Interaction between gene variants of the serotonin transporter promoter region (5-HTTLPR) and Catechol *O*-Methyltransferase (COMT) in borderline personality disorder. *American Journal of Medical Genetics Part B, 150B,* 487-495.

van Dyck, C. H., Malison, R. T., Staley, J. K., Jacobsen, L. K., Seibyl, J. P., Laruelle, M., et al. (2004). Central serotonin transporter availability measured with [^{123}I]-CIT SPECT in relation to serotonin transporter genotype. *American Journal of Psychiatry, 161,* 525-531.

Walderhaug, E. (2007). *The effects of tryptophan depletion on impulsivity in healthy men and women.* (Doctoral dissertation, University of Oslo, 2007).

Walderhaug, E., Lunde, H., Nordvik, J. E., Landrø, N. I., Refsum, H. & Magnusson, A. (2002). Lowering of serotonin by rapid tryptophan depletion increases impulsivness in normal individuals. Psychopharmacology. 164. 385-391

Walderhaug, E., Magnusson, A., Neumeister, A., Lappainen, J., Lunde, H., Refsum, H., et al. (2007). Interactive effects of sex and 5-HTTLPR on mood and impulsivity during tryptophan depletion in healthy people. *Biological Psychiatry, 62,* 593-599.

Willeit, M., Stasny, J., Pirker, W., Praschak-Rieder, N., Neumeister, A., Asenbaum, S., et al. (2001). No evidence for in vivo regulation of midbrain serotonin transporter availability by serotonin transporter promoter gene polymorphism. *Biological Psychiatry, 50,* 8-12.

World Health Organization (1993). *The ICD-10 classification of mental and behavioural disorders. Diagnostic criteria for research.* Geneve, Switzerland: World Health Organization.

Xu, X, Aysimi, E., Anney, R., Brookes, K., Franke, B., Zhou, K., et al. (2008). No association between two polymophisms of the serotonin transporter gene and combined type attention deficit hyperactivity disorder. *American Journal of Medical Genetics Part B, 147B,* 1306-1309.